Real-time PCR and PCR-tandem mass spectrometry for biodetection

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Report Documentation Page

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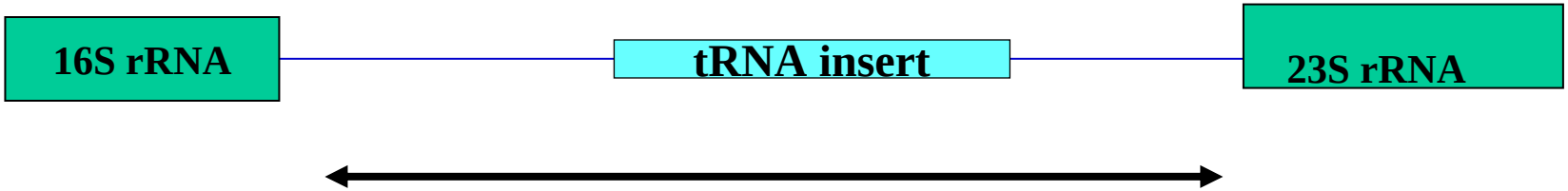
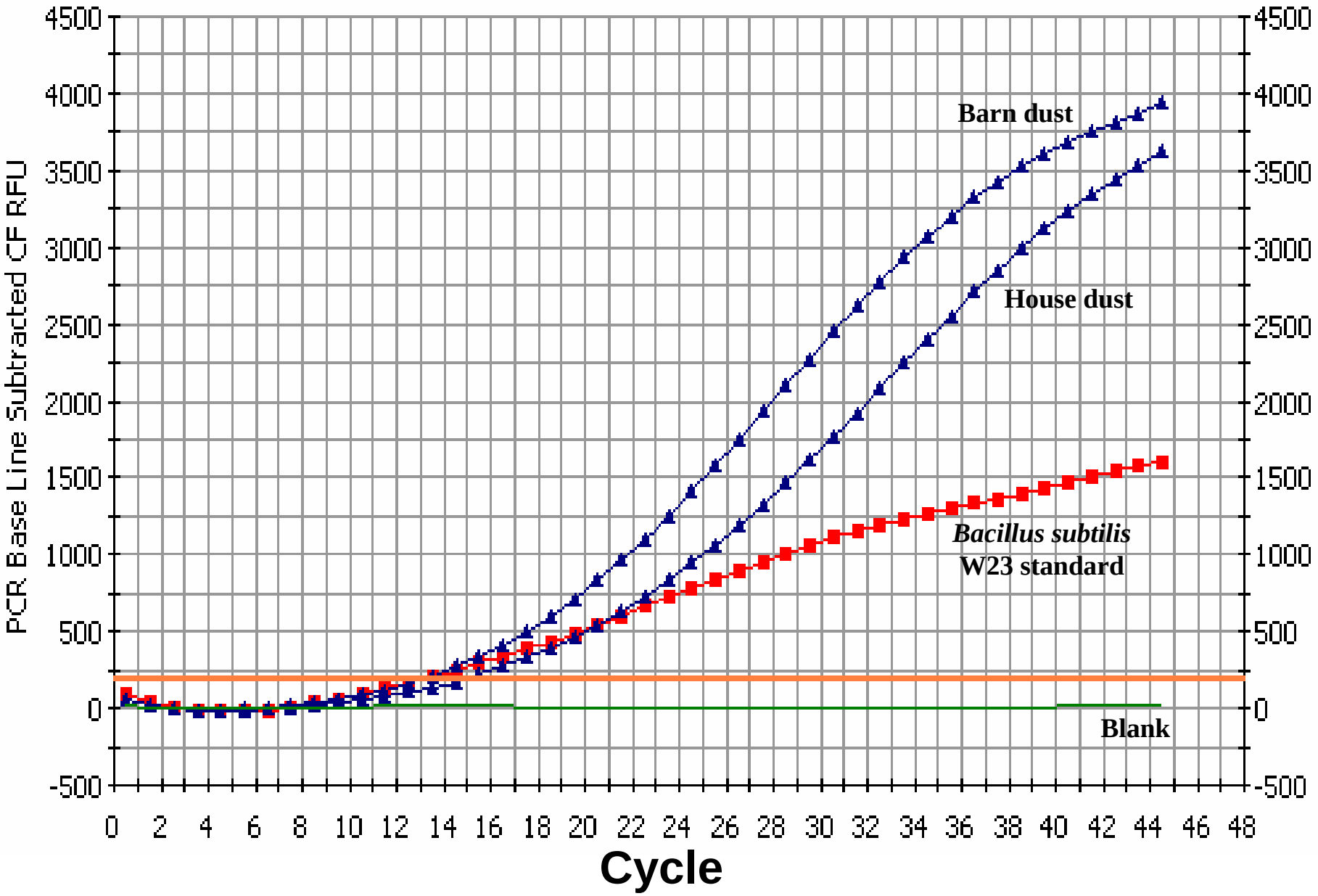
Categorizing bacilli

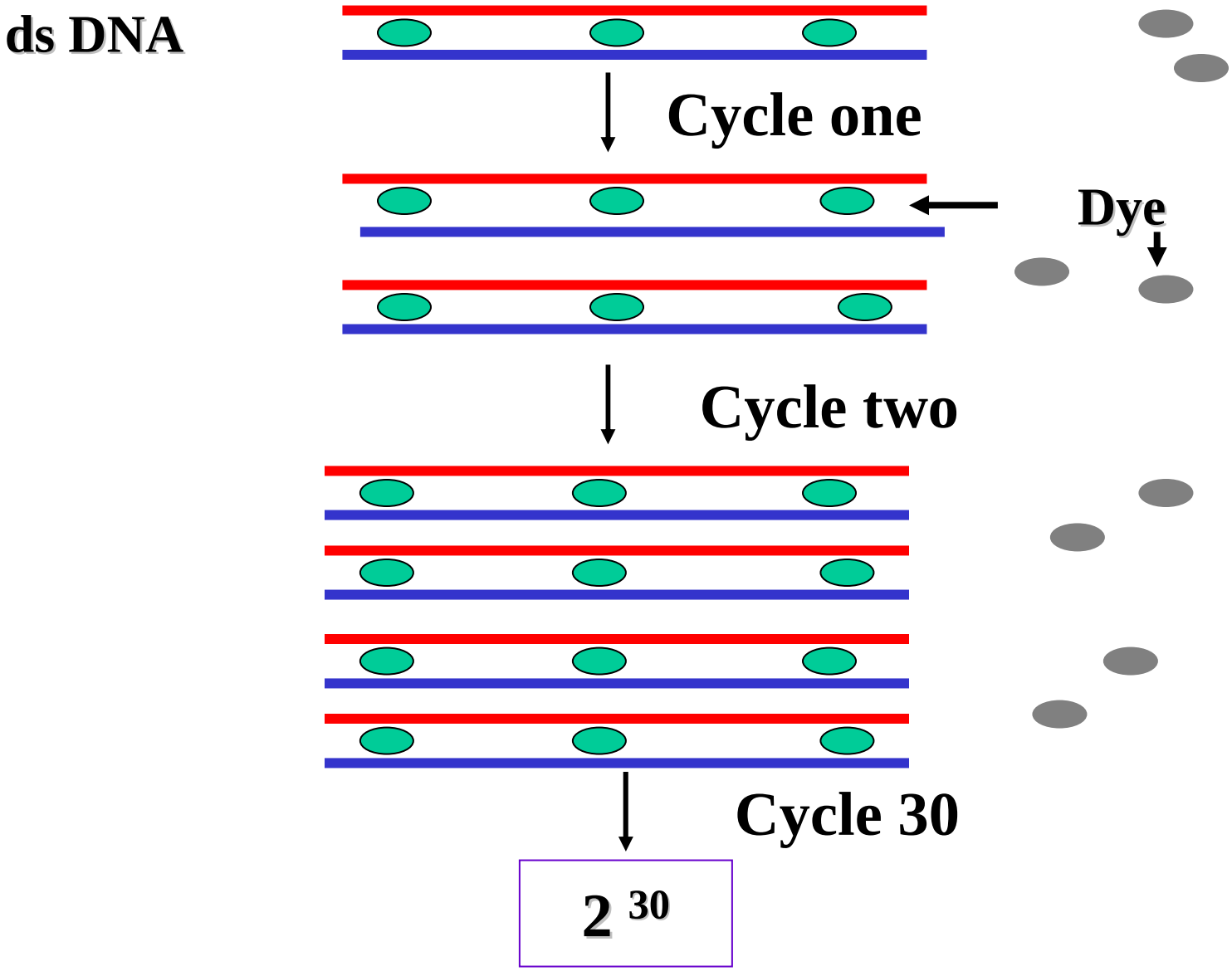


The interspace region

Real-time PCR (16s rRNA) - environmental samples

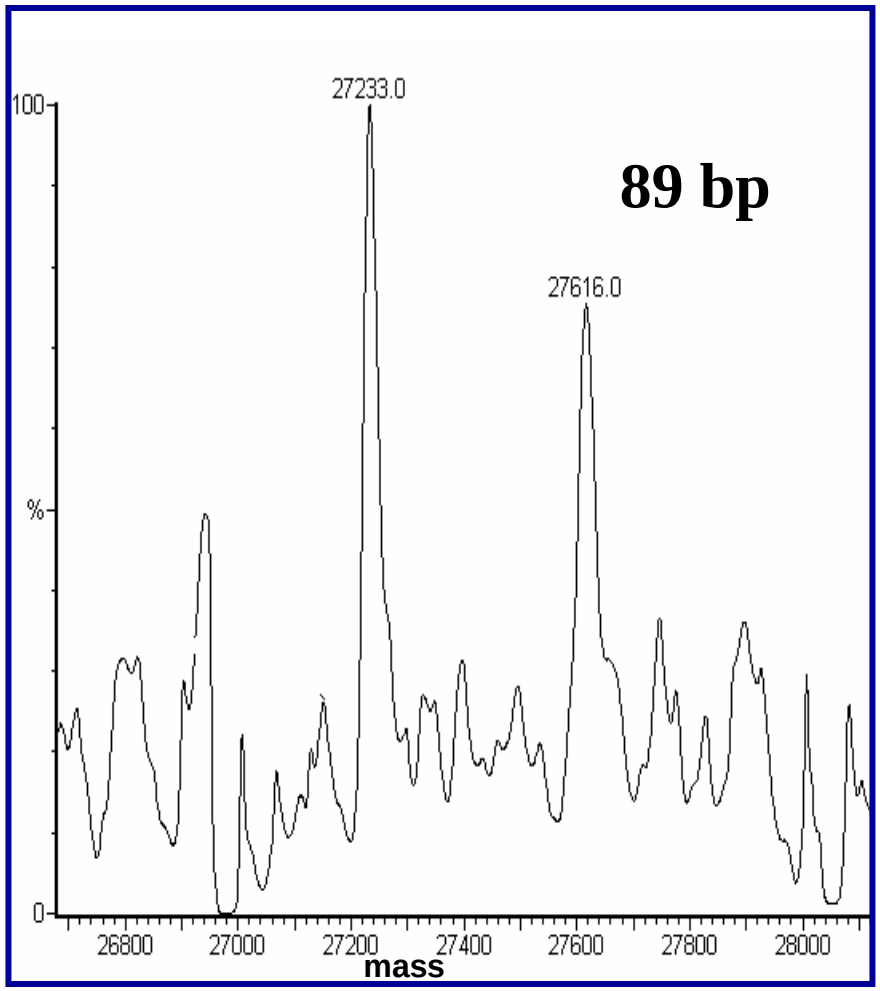


Real-time PCR

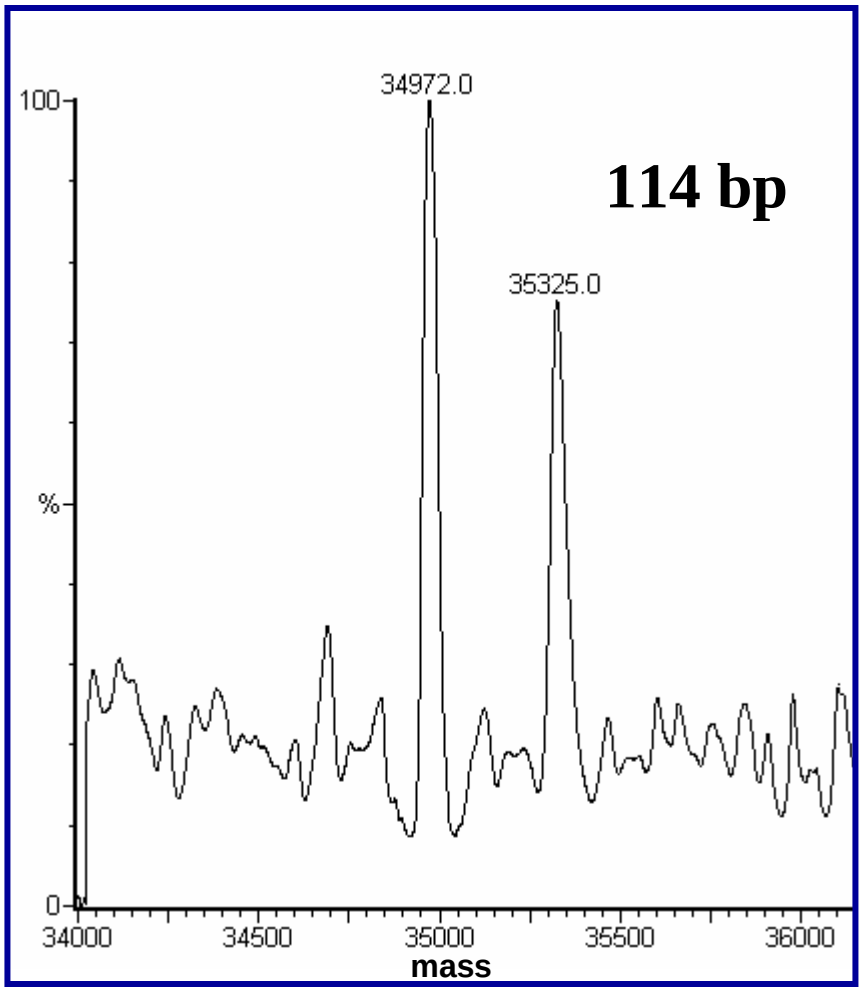


Electrospray mass spectrometry analysis of PCR products (rRNA interspace region)

Predicted Mass for *B. anthracis* (ANR-1)
Non-Coding Strand: 27618.94
Coding Strand: 27237.79



Predicted Mass for *B. subtilis* (W23)
Non-Coding Strand: 35329.01
Coding Strand: 34972.75



16S ribosomal RNA sequence

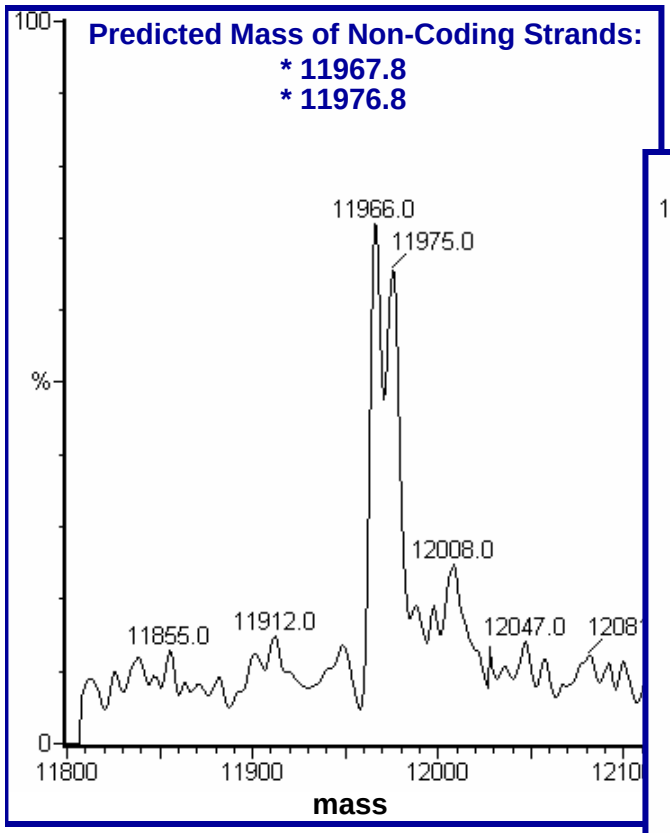
11 operons

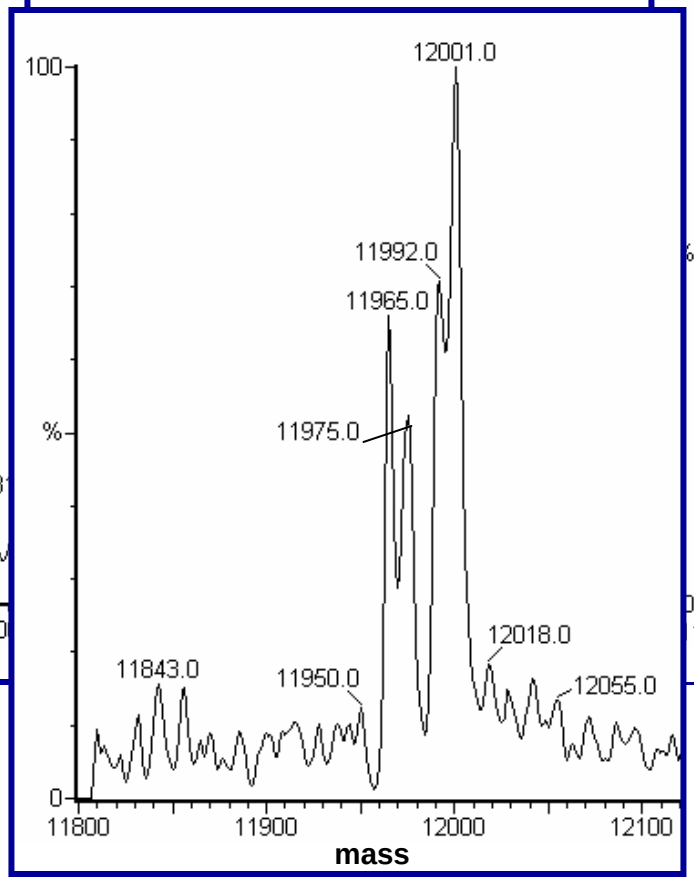
Bacillus anthracis:
6 operons contain A, 5 have T (position 1146)

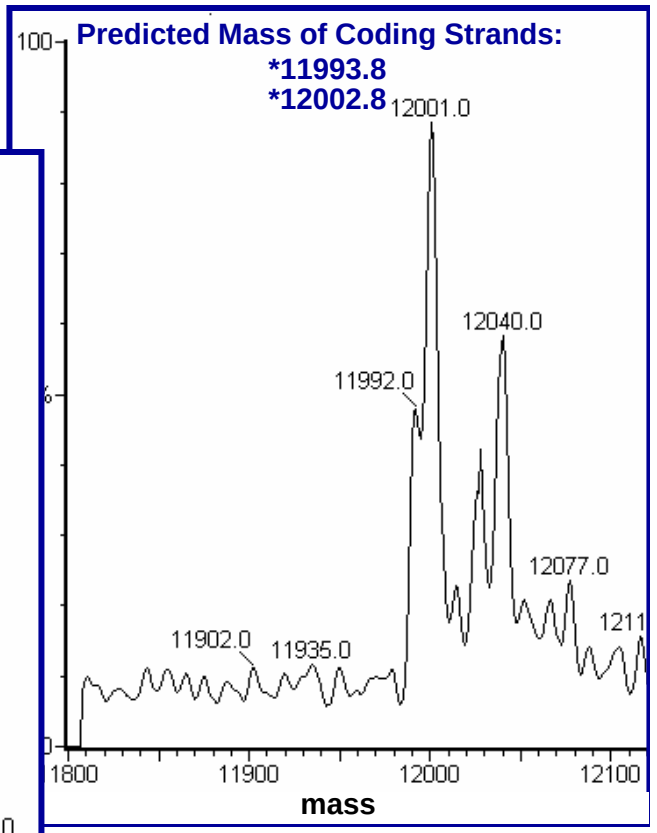
***B. cereus/B. thurinigeinsis*:**
All contain A

Sacchi et al. Emerg. Infect. Dis. 8: 1117-1123. : 2002

Molecular Weight of Two Non-Coding and Two Coding Single-Stranded 39-mers







Real-time PCR

Dust collection

DNA purification

Amplification

Detection

Under one hr

Under 2 hr

Under 7 min

Under one min

PCR-MS and MS-MS

Dust collection

DNA purification

Amplification

Clean-up

Detection

Mass spectral Data Base

PCR-Quadrupole-ESI-MS (56 bp)

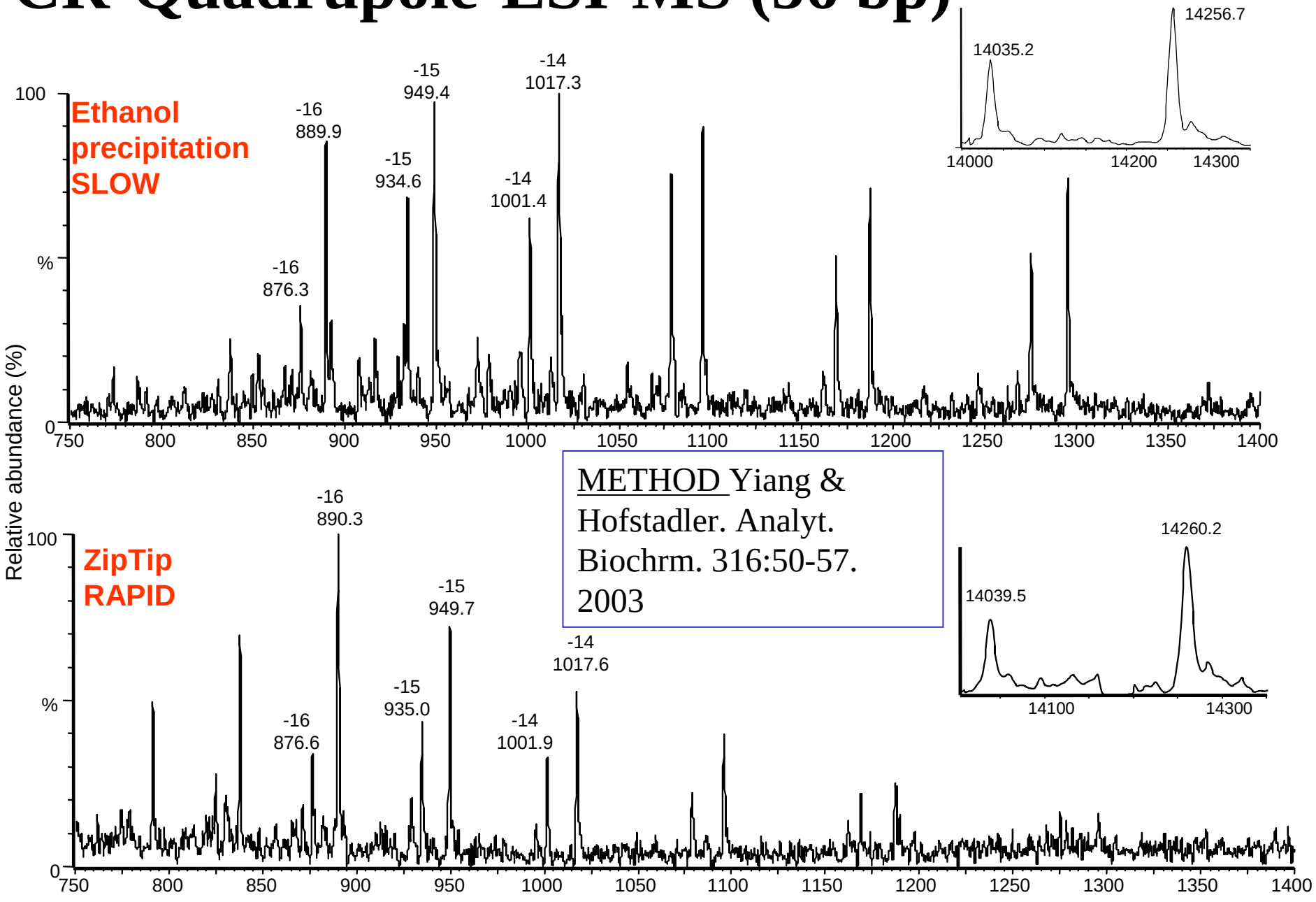
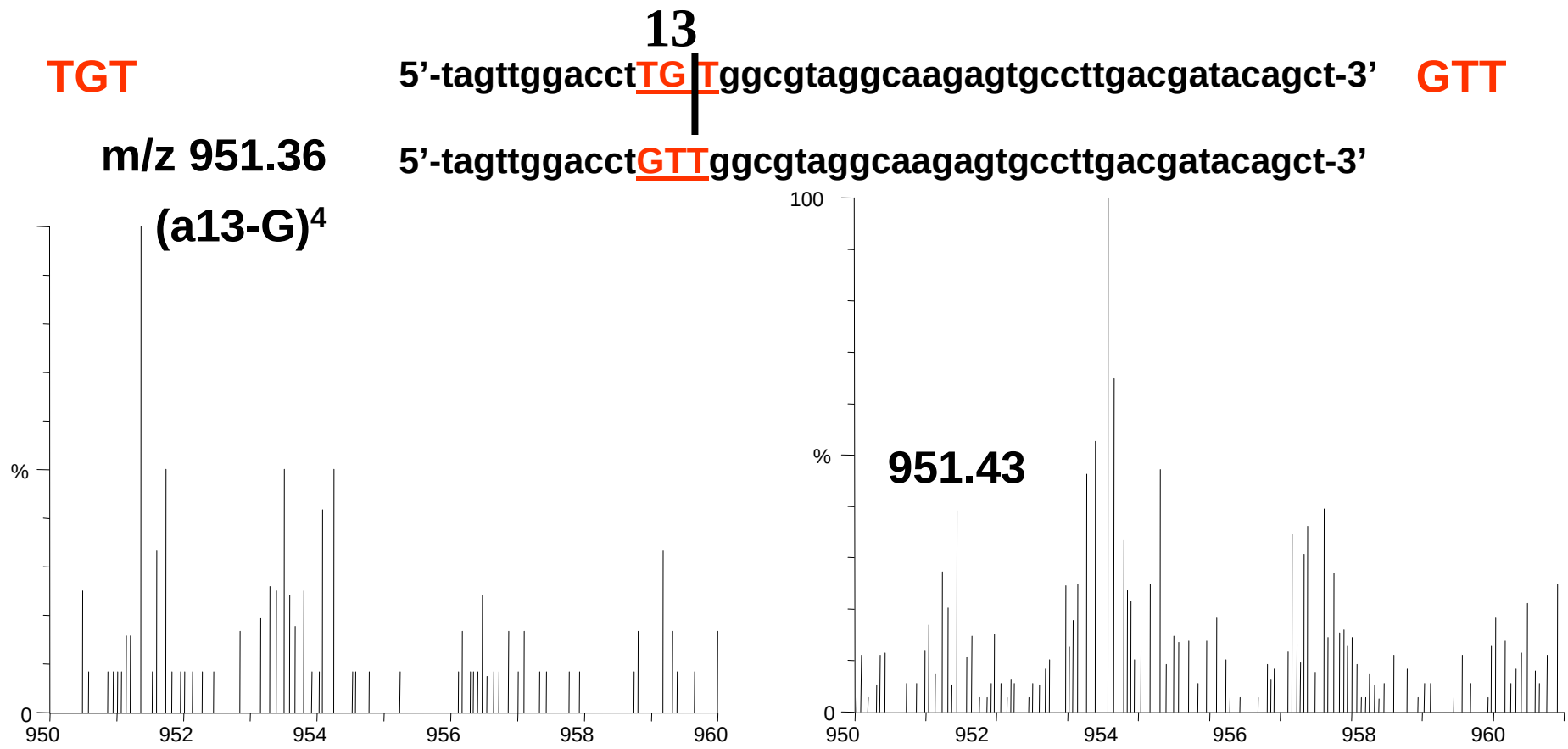


Figure 1

100 SpecDiff program differentiates pairs of MS-MS spectra of 46 bp PCR products by identifying discriminating peaks (Q-TOF)



David Tabb, Oakridge

Direct Protein detection (in comparison to PCR)

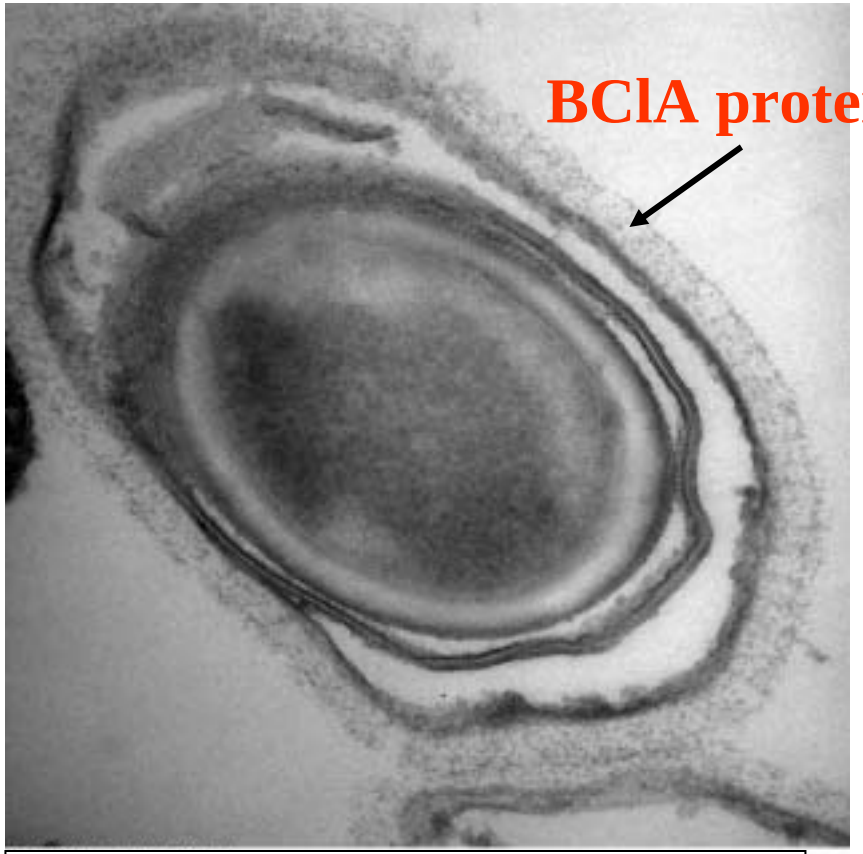
- **Potentially faster**
- **Avoids unstable biological reagents**
- **Sensitivity for environmental analysis**
  - **remains to be proven**
- **Targets need to be defined**

Carbohydrates and glycoproteins of *B.anthracis*

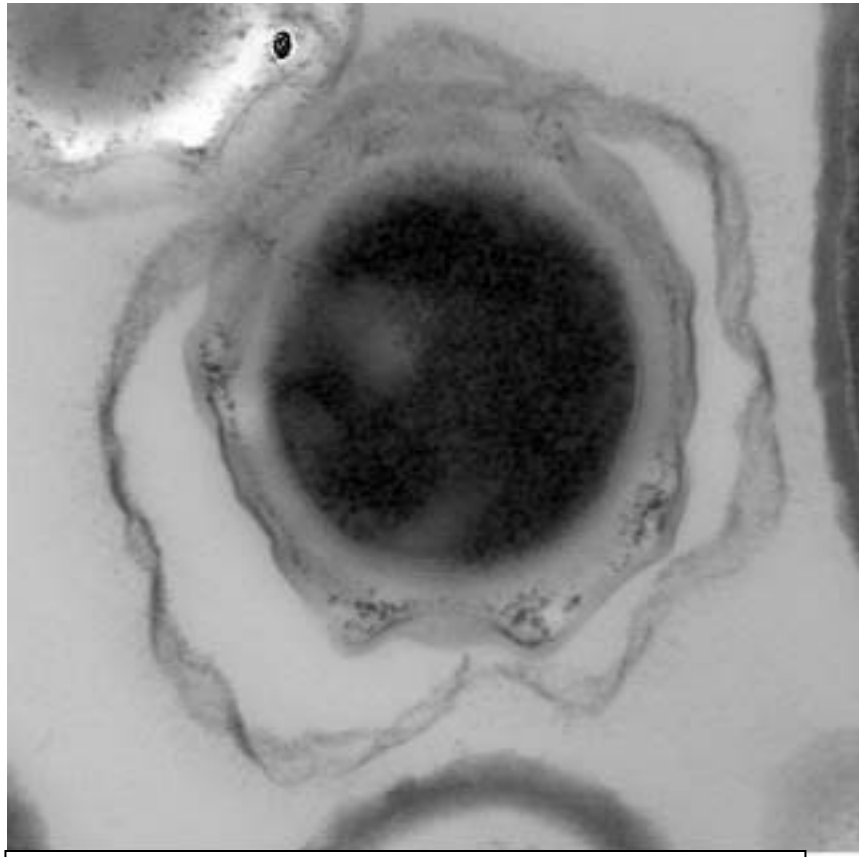
- **Spore-specific sugars**
  - **Fox et al., J. Clin. Microbiol. 31:887-894. 1993**
  - **Carbohydrate profile for *B. anthracis* distinct from *B. thuringiensis*/*B. cereus***
- **Exosporium glycoprotein, BclA**
  - **Sylvestre et. al. Mol. Microbiol. 45:169-178. 2002**

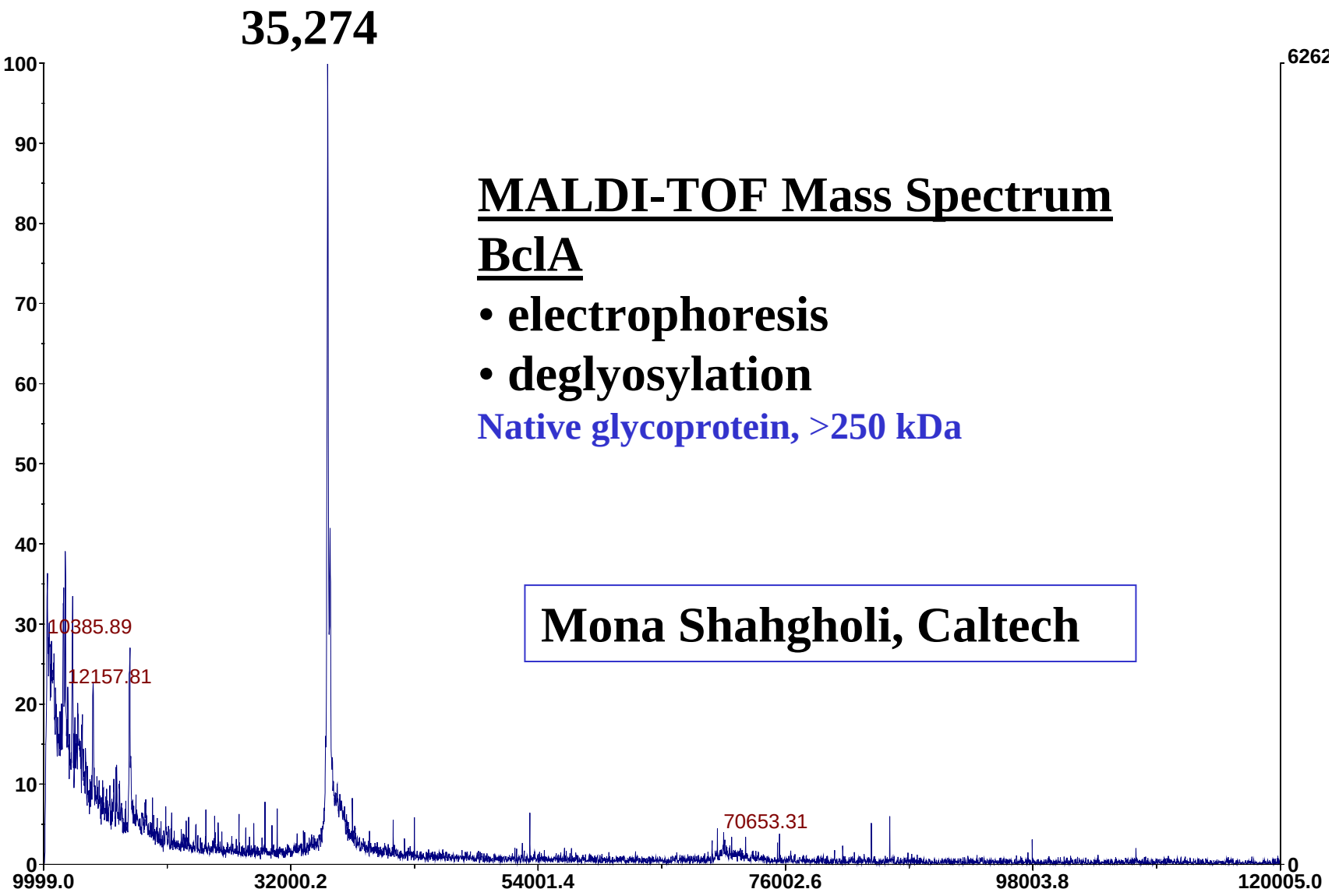
B. anthracis spore

Stained for glycoprotein



No glycoprotein stain





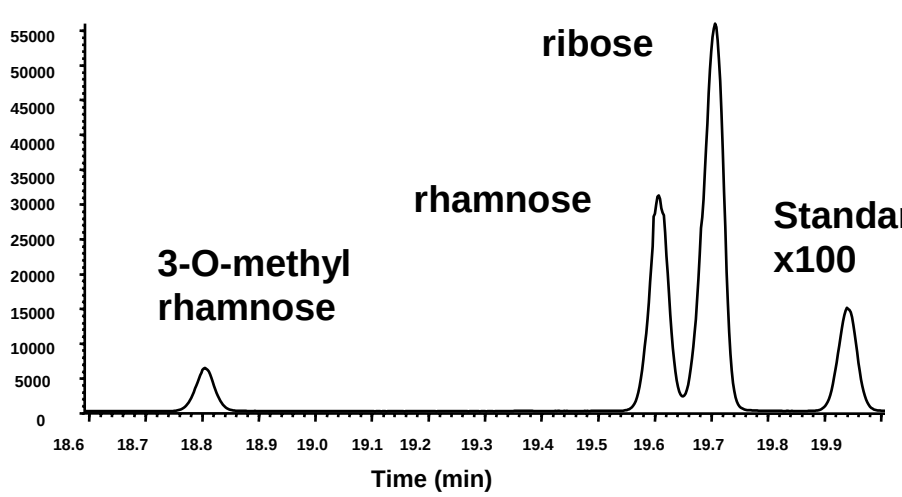
MALDI-TOF Mass Spectrum BclA

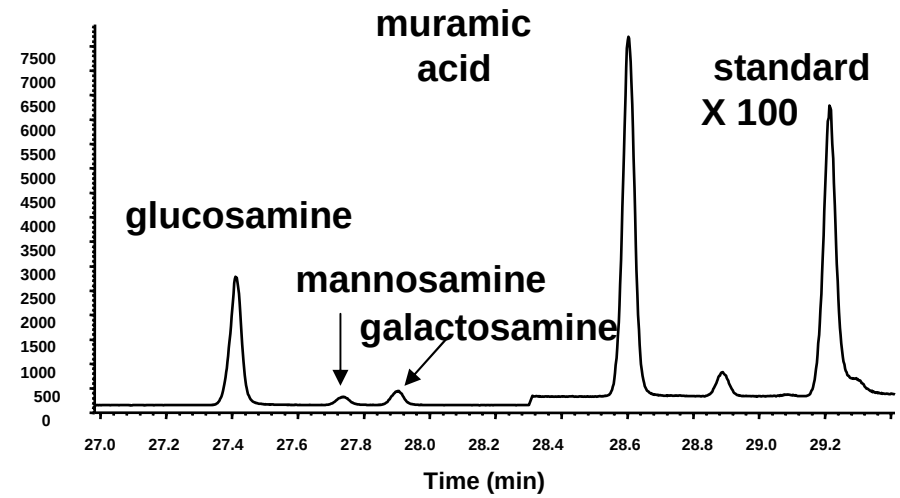
- electrophoresis
- deglyosylation

Native glycoprotein, >250 kDa

Mona Shahgholi, Caltech

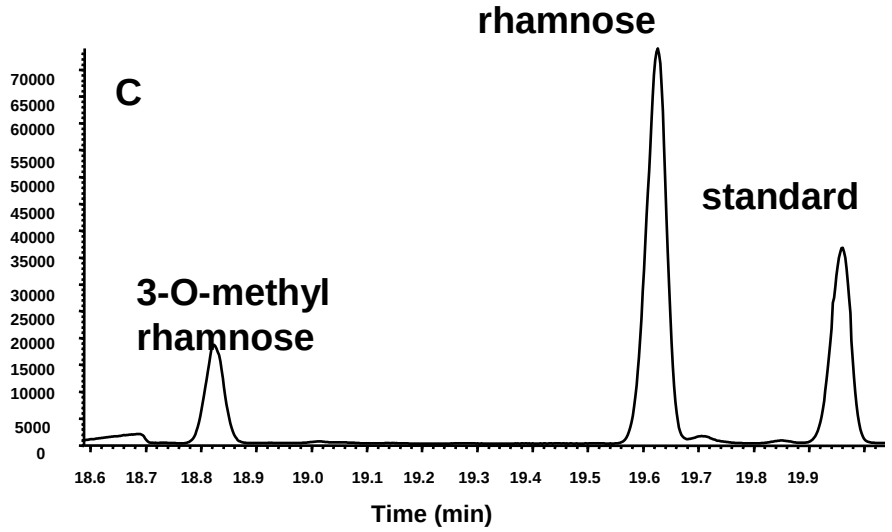
Spore

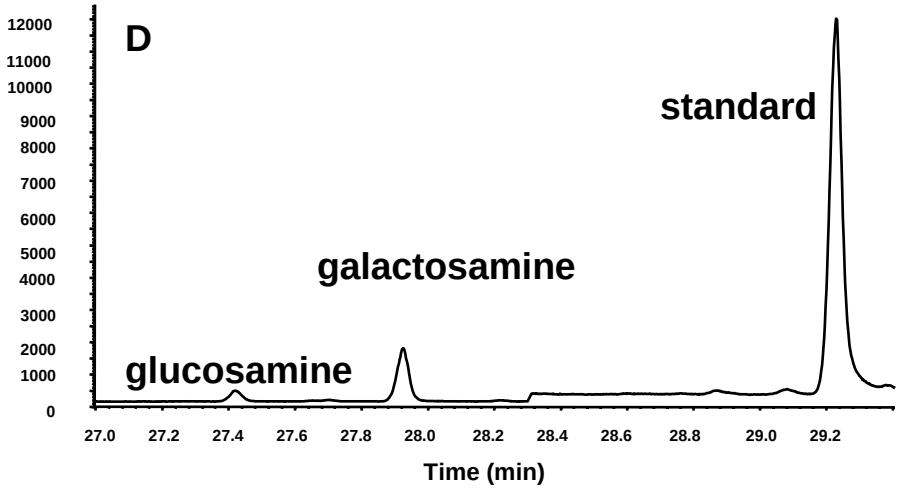




Isolated BClA

Abundance





Conclusions

- **Real-time PCR**
  - **the current gold standard**
  - **complex enzymatic reaction**
- **PCR -MS-MS**
  - **structure information**
  - **improved specificity**
- **Protein analysis (e.g. ESI or MALDI MS-MS)**
  - **potential simplicity**
  - **sensitivity to be proven for environmental monitoring**